

CLAIMS AS ALLOWED IN U.S. SERIAL NO. 09/443,067

1. An isolated nucleic acid molecule that encodes a polyphenol oxidase (PPO) polypeptide of banana, wherein said nucleic acid molecule or its complement comprises a nucleotide sequence selected from the group consisting of:
 - (i) a nucleotide sequence of: SEQ ID NO: 21;
 - (ii) a nucleotide sequence that encodes a polypeptide having the amino acid sequence of: SEQ ID NO: 22; and
 - (iii) a nucleotide sequence that is complementary of (i) or (ii).
4. The isolated nucleic acid molecule according to claim 1, wherein the PPO polypeptide of banana is at least expressed in banana peel.
7. A recombinant vector comprising the isolated nucleic acid molecule according to claim 1 inserted within a vector molecule.
8. The recombinant vector according to claim 7 wherein the vector is a plasmid expression vector.
9. The recombinant vector according to claim 8 wherein the plasmid expression vector is Bluescript SK+.
10. The recombinant vector according to claim 7 wherein the vector is a binary vector suitable for introducing said nucleic acid molecules into a plant cell, tissue or organ.
11. The recombinant vector according to claim 7, wherein the vector is replicated and the PPO-encoding nucleotide sequence is transcribed and translated in a unicellular organism or in a plant.

12. A method of increasing the level of PPO activity in a plant or a cell, tissue or organ thereof, said method comprising:
 - (a) introducing a nucleotide sequence to the plant or a cell, tissue or organ thereof which nucleotide sequence encodes a PPO polypeptide of banana and is selected from the group consisting of:
 - (i) a nucleotide sequence having the sequence of: SEQ ID NO: 21;
 - (ii) a nucleotide sequence that encodes a polypeptide having the amino acid sequence of: SEQ ID NO: 22; and
 - (iii) a nucleotide sequence that is complementary to (i) or (ii), and
 - (b) expressing the nucleotide sequence to produce an enzymatically-active PPO polypeptide.
13. A method of introducing into a banana plant or a cell, tissue or organ thereof a nucleic acid molecule which hybridizes to a nucleotide sequence in the banana plant, cell, tissue or organ thereof selected from the group consisting of:
 - (i) a nucleotide sequence having the sequence of: SEQ ID NO: 21;
 - (ii) a nucleotide sequence that encodes a polypeptide having the amino acid sequence of: SEQ ID NO: 22; and
 - (iii) a nucleotide sequence that is complementary to (i) or (ii),comprising contacting the banana plant or the cell, tissue or organ thereof with the nucleic acid molecule.
14. The method according to claim 13 further comprising expressing the introduced nucleic acid molecule to produce sense or antisense RNA therefrom.

17. The method according to claim 12 wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of *Agrobacterium*-mediated transformation.
18. The method according to claim 13 wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of *Agrobacterium*-mediated transformation.
19. The method according to claim 12, wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of microparticle bombardment using a nucleic acid-coated microprojectile.
20. The method according to claim 13, wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of microparticle bombardment using a nucleic acid-coated microprojectile.
21. A transformed plant comprising the isolated nucleic acid molecule according to claim 1 or a plant part, progeny or propagule thereof that also comprises said nucleic acid molecule.
22. A transformed plant comprising the recombinant vector according to claim 7, or a plant part, progeny or propagule thereof that also comprises said nucleic acid molecule.
23. A process for isolating a nucleic acid molecule from banana the process comprising:
 - (i) providing:
 - (a) banana cells, tissue or organs expressing the protein of SEQ ID NO: 22;
 - (b) a first primer having a nucleotide sequence which hybridizes to SEQ ID NO: 21;

(c) a second primer having a nucleotide sequence which hybridizes to the complement of SEQ ID NO: 21

- (ii) isolating RNA from said cells, tissues or organs;
- (iii) treating the RNA to construct copy DNA (cDNA) therefrom; and
- (iv) amplifying the cDNA so formed using the first and second primers.

27. The process of claim 23 further comprising obtaining a nucleic acid encoding the N-terminal fragment of the PPO polypeptide of banana by attaching an anchor to the 5'-end of the cDNA formed and amplifying said cDNA using a first primer that binds to said anchor and a second primer in the antisense orientation, and wherein the nucleotide sequence of said second primer is derived from the internal sequence of the amplified cDNA.

28. The process of claim 23 further comprising obtaining a nucleic acid encoding the C-terminal fragment of the PPO polypeptide of banana by amplifying said cDNA using an adaptor primer and a second primer in the sense orientation, and wherein the nucleotide sequence of said second primer is derived from the internal sequence of the amplified cDNA.

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In the claims

Please cancel claim 1-33 without prejudice to pursue the subject matter of these claims in a related application.

Please add the following new claims 34-55 as follows:

34. (New) An isolated nucleic acid molecule that encodes a banana polypeptide having polyphenol oxidase (PPO) activity, or a complement of the nucleic acid molecule.

35. (New) The isolated nucleic acid molecule of claim 34, wherein the molecule has

- a) a nucleic acid sequence set forth in SEQ ID NO 1, 3, 5, 7 or 23;
- b) a nucleic acid sequence that encodes a polypeptide having the amino acid sequence set forth in SEQ ID NO 2, 4, 6, 8 or 24; or
- c) a nucleic acid sequence that encodes a cooper-binding site of the amino acid sequence in (b).

36. (New) The isolated nucleic acid molecule of claim 34, wherein the banana polypeptide is at least expressed in banana peel.

37. (New) A recombinant vector comprising the nucleic acid molecule of claim 34 linked within a vector molecule.

38. (New) The recombinant vector of claim 37 wherein the vector is a plasmid expression vector.

39. (New) The recombinant vector of claim 38 wherein the plasmid expression vector is Bluescript SK+.

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40. (New) The recombinant vector of claim 37 wherein the vector is a binary vector suitable for introducing into a plant cell, tissue or organ.
41. (New) The recombinant vector of claim 37, wherein the vector is capable of being replicated and the PPO-encoding nucleic acid is capable of being transcribed and translated in a unicellular organism or in a plant.
42. (New) A transformed plant, plant part, progeny or propagule thereof, comprising a nucleic acid molecule that encodes a banana polypeptide having polyphenol oxidase (PPO) activity, or a complement of the nucleic acid molecule.
43. (New) A transformed plant, plant part, progeny or propagule thereof, comprising the recombinant vector of claim 37.
44. (New) A method of increasing the level of PPO activity in a plant or a cell, tissue or organ thereof, said method comprising:
- (a) introducing a nucleotide sequence to the plant or a cell, tissue or organ thereof which nucleotide sequence encodes a PPO polypeptide of banana, lettuce, tobacco or pineapple and having
 - (i) a nucleotide sequence set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27 or 29;
 - (ii) a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 28, or 30;
 - (iii) a nucleotide sequence that encodes a copper-binding site of the amino acid sequence of (ii);
or
 - (iv) a nucleotide sequence that is complementary to

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- (i) or (ii) or (iii), and
- (b) expressing said nucleotide sequence to produce an enzymatically-active PPO polypeptide.
45. (New) A method of decreasing the level of PPO activity in a banana, lettuce, pineapple or tobacco plant or a cell, tissue or organ thereof, the method comprising introducing a nucleic acid molecule which hybridizes to a nucleotide sequence in the banana, lettuce, pineapple or tobacco plant or a cell, tissue or organ thereof, having
- (i) a nucleotide sequence set forth in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27, or 29;
 - (ii) a nucleotide sequence that encodes an amino acid sequence set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 28, or 30;
 - (iii) a nucleotide sequence that encodes a copper-binding site of the amino acid sequence in (ii); or
 - (iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii).
46. (New) A process for isolating a nucleic acid molecule that encodes a PPO polypeptide of banana, lettuce, tobacco or pineapple, the method comprising:
- (i) providing:
 - (a) banana, lettuce, tobacco or pineapple PPO cells, tissue or organs having PPO activity,
 - (b) a first primer having a nucleotide sequence capable of hybridizing to a copper (Cu) binding site-encoding region of a PPO gene selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27, and 29 or

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upstream thereof;

(c) a second primer having a nucleotide sequence capable of hybridizing to the complement of a copper (Cu) binding site-encoding region of a PPO gene selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 23, 25, 27, and 29, or downstream thereof; and

(d) an adaptor primer;

(ii) isolating RNA from said cells, tissues or organs;

(iii) treating the RNA to construct copy DNA (cDNA) therefrom; and

(iv) amplifying the cDNA so formed using the first and second primers.

47. (New) The process of claim 46 wherein the first primer comprises a nucleotide sequence selected from the group consisting of:

(i) 5'-GCGAATTCTT[TC][TC]TICCITT[TC][CA][TC][AC]G-3' (SEQ ID NO: 31);

(ii) 5'-GCGAATTCGATCCCIACITT[TC]GC[GT]TTICC-3' (SEQ ID NO: 32);

(iii) 5'-GCGAATTCAA[TC]GTIGA[TC][AC]GIATGTGG-3' (SEQ ID NO: 33);

(iv) 5'-GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3' (SEQ ID NO: 34);

(v) 5'-GCGAATTCTTICCIT[TA][TC]TGGAA[TC]TGGG-3' (SEQ ID NO: 35); and

(vi) a hybridizable fragment of any one of (i) to (v).

48. (New) The process of claim 46 wherein the second primer comprises a nucleotide sequence:

(i) 5'-GCCTGCAGCCAGATIC[TG][AG]TCIAC[AG]TT-3' (SEQ ID NO: 36); or

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(ii) 5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3' (SEQ ID NO: 37).

49. (New) The process of claim 46 wherein treating the RNA to construct cDNA includes treating the RNA with reverse transcriptase and an adaptor primer that comprises the nucleotide sequence:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTTTTTT-3' (SEQ ID NO:

38) or a hybridizable fragment thereof to form cDNA.

50. (New) The process of claim 46 wherein the process further comprises obtaining nucleic acid encoding the N-terminal fragment of the PPO polypeptide of banana, lettuce, tobacco or pineapple by attaching an anchor to the 5'-end of the cDNA formed and amplifying said cDNA using a first primer that binds to said anchor and a second primer in the antisense orientation, and wherein the nucleotide sequence of said second primer is derived from the sequence of the internal PPO fragment.

51. (New) The process of claim 50 wherein the primer in the antisense orientation comprises a nucleotide sequence:

(i) 5'-ATATCACCTGTCGGTACATGACGGC-3' (SEQ ID NO: 39);

(ii) 5'-GTGCCATTGTAGTCGAGGTCAATCA-3' (SEQ ID NO: 40); or

(iii) 5'-CCAGTGCCTGGTTTGGTGTATTCAC-3' (SEQ ID NO: 41).

52. (New) The process of claim 46 wherein the process further comprises obtaining nucleic acid encoding the C-terminal fragment of the PPO polypeptide of banana, lettuce, tobacco or pineapple by amplifying said cDNA using an adaptor primer and a primer in the sense orientation, and wherein the nucleotide sequence of said second primer is derived from the sequence of the internal PPO fragment.

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53. (New) The process of claim 52 wherein the primer in the sense orientation comprises the nucleotide sequence:

5'CGCTGGGTGGGTAATTCTAGGATG-3' (SEQ ID NO: 46);

5'-AGTCATCCACAATGCGGCGCACATG-3' (SEQ ID NO: 47); or

5'-GTTGCTCTTCTTAGGCTCGGCTTAC-3' (SEQ ID NO: 48).

54. (New) The process of claim 52 wherein the adaptor primer includes the following sequence or a hybridizable fragment thereof:

5'-GACTCGAGTCGACATCG-3' (SEQ ID NO: 49).

55. (New) An isolated nucleic acid molecule that encodes a PPO polypeptide of lettuce, tobacco or pineapple wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence selected from group consisting of: SEQ ID NOS: 9, 11, 13, 15, 17, 19, 25, 27, and 29;
- (ii) a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of: SEQ ID NOS: 10, 12, 14, 16, 18, 20, 26, 28, and 30;
- (iii) a nucleotide sequence that encodes a cooper-binding site of an amino acid sequence of (ii); and
- (iv) a nucleotide sequence that is complementary to (i) or (ii) or (iii).